Review Article

Blood-Ocular Barriers

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Article info

Abstract

There are two main blood-ocular barriers, the blood-aqueous barrier and the blood-retinal barrier. The blood-aqueous barrier is formed by the non-pigmented ciliary epithelium of the ciliary body and the vascular endothelium of the iris vessels. The blood-retinal barrier is formed by the vascular endothelium of the retinal vessels and the retinal pigment epithelium. Four methods of examination are currently used to study the function of blood-ocular barriers. Among these, laser flare-cell photometry is a non-invasive, quantitative method to evaluate the permeability of the blood-aqueous barrier. Vitreous fluorophotometry is an excellent technique to quantitate blood-retinal barrier function. Fluorescein angiography and optical coherence tomography are excellent qualitative imaging techniques to evaluate blood-retinal barrier function. Current basic research shows prostaglandin E_2 and other mediators may produce breakdown of the blood-aqueous barrier, and vascular endothelial growth factor plays an important role in the breakdown of the blood-retinal barrier. Retinal laser photocoagulation can induce breakdown of both the blood-aqueous and blood-retinal barriers in pigmented rabbits. The four methods of examination described herein are excellent measures for clinical application to evaluate blood-ocular barrier function in various ocular diseases, many of which are discussed here. (*Tzu Chi Med J* 2008;20(1):25–34)

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1.   Introduction

The normal adult eye is approximately spherical in shape, with an anteroposterior diameter averaging 24.2 mm along the geometrical axis. The eye is composed of a three-layered eye wall and an interior portion. The eye wall consists of an outer fibrous layer (including the cornea and sclera); a middle vascular layer (including the iris, ciliary body and choroid); and an inner nerve tissue layer (the retina). The ocular vasculature includes the central retinal artery, which supplies the retina, and ciliary vessels, which supply the outer and middle layers of the eye wall.

The interior portion of the eye is composed of the aqueous, lens and vitreous. The aqueous is a clear fluid that fills the anterior and posterior chambers

of the eye. Aqueous is produced by the ciliary body and is secreted into the posterior chamber. It passes through the pupil into the anterior chamber, and then spreads peripherally toward the angle of the anterior chamber. The vitreous is a transparent, avascular, inert gel that comprises two-thirds of the eye. It is 99% water, with the remaining 1% made up of collagen and hyaluronic acid.

The barrier system separates the interior portion of the eye from blood entering the eye, and maintains the transparency and function of the interior portion of the eye. There are two main blood-ocular barriers, the blood-aqueous barrier and the blood-retinal barrier [1].

2.　　Anatomy and physiology

2.1.　The blood-aqueous barrier

There are two main structures involved in the formation of the blood-aqueous barrier (BAB), the ciliary body and the iris (Fig. 1). The nonpigmented ciliary epithelium of the ciliary body produces the aqueous (the fluid in the anterior and posterior chambers). The components of the aqueous are quite different from those plasma exudates present in the ciliary body stroma. There are different concentrations of electrolytes, other small molecules, and a restricted set of proteins in low concentrations between the aqueous and the plasma. A barrier to the free diffusion of molecules is formed by tight junctions between the nonpigmented ciliary epithelial cells. This type of barrier is an epithelial barrier. In the iris, tight junctions between the vascular endothelial cells contain proteins similar to the epithelial tight junctions. This type of barrier in the iris vessels is also an endothelial barrier [2].

The BAB contributes to the nutrition and function of the cornea and the lens. Alterations in the BAB may occur in ocular inflammation, intraocular surgery, trauma, or vascular diseases. The aqueous becomes cloudy due to leakage of plasma proteins into the posterior and anterior chambers. The aqueous may become plasmoid due to the presence of fibrinogen and other proteins. Inflammatory cells may also be present when breakdown of the BAB occurs.

2.2.　The blood-retinal barrier

The retina has two areas of direct interaction with the blood, one at the level of the retinal vessels and the other at the level of the choroid-retinal pigment epithelial interface. Thus, two structures comprise the blood-retinal barrier (BRB). The BRB is formed by tight junctions between the endothelial cells of the retinal
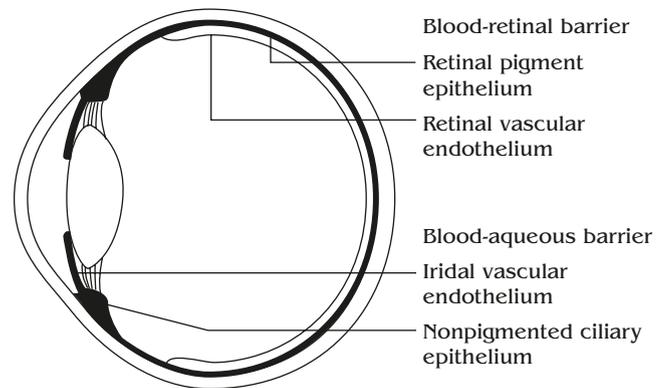

Fig. 1 — **Anatomy of blood-ocular barriers. The blood-aqueous barrier is formed by the vascular endothelium of the iris vessels and the nonpigmented ciliary epithelium of the ciliary body. The blood-retinal barrier is formed by the vascular endothelium of the retinal vessels and the retinal pigment epithelium.**

vessels (the inner BRB) and by similar tight junctions in the retinal pigment epithelium (the outer BRB). The inner BRB makes the retinal vessels impermeable to molecules larger than 20–30 kDa. Small molecules, such as glucose and ascorbate, are transported by facilitated diffusion. In the outer BRB, the retinal pigment epithelial cells are aligned by tight intercellular junctional complexes termed zonula occludens. Bidirectional transport may occur with various metabolites such as glucose. The bulk flow of fluid from the retinal side to the choriocapillaries is carried out through mechanisms such as the Na^+/K^+ ATPase pump and by transport of nonionic solutes such as amino acids and glucose.

In conclusion, there are two types of molecular movement across the BRB. Active transport takes place across the BRB, mostly from the vitreous into the blood. It has been shown to occur with amino acids, a variety of organic anions, prostaglandins, and fluorescein. Passive transfer across the BRB and into the vitreous due to the concentration gradient is extremely limited, but does occur with sodium, phosphate, glucose and potassium [3].

3.　　Methods of examination

3.1.　Laser flare-cell photometry

The laser flare-cell meter is a new, noninvasive instrument that can be used to quantitatively evaluate the permeability of the BAB by measuring the protein concentration and number of cells in the anterior chamber. The instrument is composed of a He-Ne laser beam system (25 μW intensity, 20 μm diameter), a photomultiplier mounted on a slit-lamp microscope, and a personal computer which controls

A

B

Fig. 2 — Laser flare-cell meter: (A) equipment; (B) recorded data.

the system and analyzes the data detected by the photomultiplier. A coaxial halogen illumination light is used for observation, but it is turned off during measurement by means of a synchronized lens shutter. In practice, the He-Ne laser beam is projected into the center of the anterior chamber of the eye by means of an optical scanner. The laser light scattered by protein and the light reflection from the cells in the anterior chamber is passed through a sampling window (0.3×0.5mm) in the microscope. The intensity of the scattered light collected through the sampling window is detected by the photomultiplier, and then the data are analyzed by the personal computer (Fig. 2A) [4,5].

The instrument has two measurement modes, one for protein concentration and the other for cell counts (Fig. 2B).

3.1.1. Protein concentration measurement mode

The laser beam is scanned vertically for 0.6mm which covers the sampling window. The intensity of the light scattered by proteins in the anterior chamber is detected by the photomultiplier when the laser beam is passed through the sampling window. Measurements are also taken when the laser beam passes above and below the sampling window to detect the background signal. The average of the background measurements is subtracted from the data obtained in the scanned sampling window to provide protein measurements. The aqueous flare, representing protein in the aqueous, is expressed in photon count/msec.

3.1.2. Cell count mode

The laser beam in a Kowa FC-1000 laser flare-cell meter (Kowa, Tokyo, Japan) is scanned through a volume of aqueous of $0.5 \times 0.6 \times 0.25$ mm (0.075 mm^3) over 0.5 seconds through the sampling window. The recently developed FC-2000 scans a volume of 0.5 mm^3 for 0.7 seconds. A strong peak of reflected light is obtained when the laser beam strikes a cell in the aqueous, and the number of peaks is counted by the computer. The peaks in the scattered light are counted as the number of cells in the aqueous.

3.2. Vitreous fluorophotometry

Vitreous fluorophotometry is an excellent technique to quantitate BRB function. Basically, within 60 minutes after intravenous injection of sodium fluorescein, fluorophotometry is performed to measure the concentration of free fluorescein in the vitreous and in the plasma. The simplest measurement of the degree of BRB function is the fluorescein concentration 3mm from the retina 60 minutes after fluorescein administration. A more detailed measurement is estimation of the BRB permeability, which also requires estimation of the decay of the plasma fluorescein concentration. By comparing the concentrations of free fluorescein in these two compartments, the penetration ratio of the BRB is determined [6–8].

After administration, fluorescein diffuses into the vitreous of the normal eye from two routes, anteriorly, from the ciliary body and iris vessels, and posteriorly, from the retinal and choroidal vessels through the BRB. In a normal eye with an intact BRB, permeability to fluorescein is limited. In contrast, breakdown of the BRB increases BRB permeability, allowing fluorescein to diffuse into the vitreous at a much faster rate and in larger amounts.

The Fluorotron Master (Coherent Inc., Palo Alto, CA, USA) is the standard instrument for vitreous fluorophotometry. This instrument is basically an optical fluorophotometer composed of an optoelectronic unit and computerized system for data acquisition and processing (Fig. 3A). The excitation of fluorescein is induced by a conventional halogen lamp filtered to match the excitation spectrum of free fluorescein. Fluorescein emission is detected by a photomultiplier
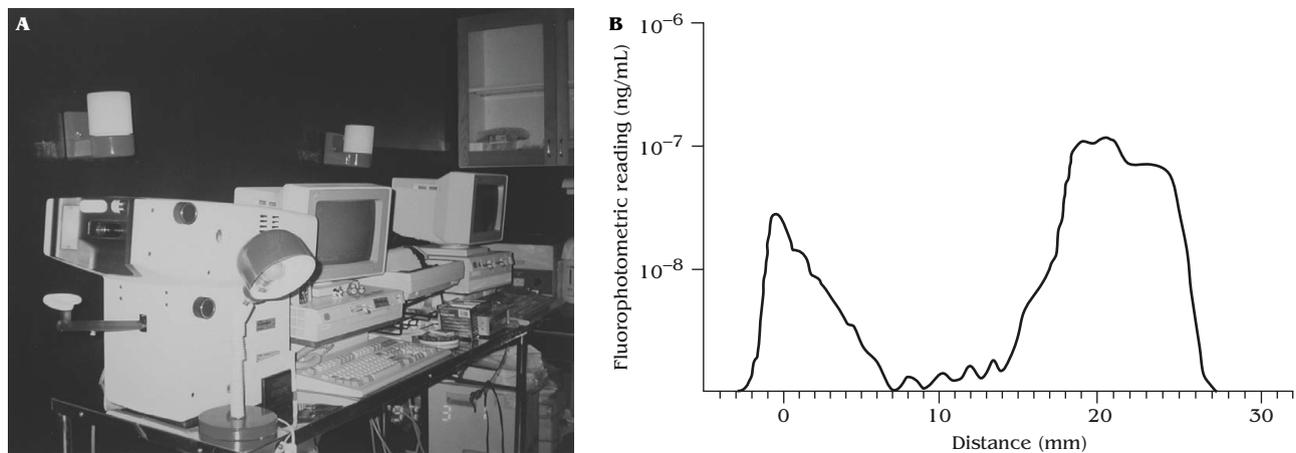
Fig. 3 — Vitreous fluorophotometry: (A) equipment; (B) normal scan.

equipped with a barrier filter. The photomultiplier then converts the light counts into electronic circuit counts. Scans are obtained before and after injection of fluorescein, and are displayed on a screen in concentration units versus distance from the retina (Fig. 3B) [6–8].

Vitreous fluorophotometry is performed according to the recommended protocol of the reference *Manual of Ocular Fluorometry* published by EUROEYE, a concerted action for standardization of ocular fluorometry. Briefly, 20% sodium fluorescein is first injected intravenously at a dose of 14 mg/kg. Fluorophotometric scans are performed along the optical axis from the retina to the cornea using the posterior segment program on both eyes. These scans are taken before fluorescein injection, 3–5 minutes after injection, and 60 minutes after injection. Blood samples taken from the non-injected arm at 10, 30 and 50 minutes are placed into tubes containing EDTA and subsequently centrifuged at 3000 rpm for 15 minutes. The plasma is then ultrafiltered using Amicon hydrophilic anisotropic membranes. A fluorophotometric scan is then performed on the diluted ultrafiltrates, measuring the concentration of free fluorescein with an optimized protocol to measure the BRB penetration ratio.

The penetration ratio of fluorescein into the vitreous is measured according to the following formula:

$$PR = PV_{3\,mm}/Plasma\,fluorescein\,integral_{(3\,min-60\,min)}$$ in which PR represents the penetration ratio, and $PV_{3\,mm}$ represents the average value determined 3 mm in front of the choroid retinal peak. The plasma fluorescein integral is calculated by standard software.

3.3.   *Fluorescein angiography*

Fluorescein angiography is a qualitative imaging technique to evaluate BRB function using a fundus camera to photograph the passage of fluorescein in the retinal and choroidal circulation after intravenous injection. Sodium fluorescein absorbs blue light (465–490 nm) and emits green light (520–530 nm). However, when sodium fluorescein is injected intravenously, about 80–90% of the dye is bound to plasma protein and is not available for fluorescence. Only the remaining unbound or free fluorescein is available to contribute to fluorescence during the procedure.

A fundus camera equipped with both excitation and barrier filters is used to perform fluorescein angiography. A blue excitation filter is placed in front of the light beam of the fundus camera, allowing only blue light to enter the eye. The free fluorescein molecules in the ocular blood vessels are struck by the blue light, absorb it, and then emit green light. The emitted green light passes through the green barrier filter, and is captured on film in the fundus camera [9,10].

In fluorescein angiography, the pupils are first dilated with topical 1% tropicamide and 10% phenylephrine solution. The patient is seated in front of the fundus camera. Initially, color fundus photographs are taken. Fluorescein solution is then quickly injected intravenously after inserting both the blue excitation and green barrier filters into the fundus camera. Photographs are then taken at intervals.

In the normal fluorescein angiogram, the fluorescein dye first appears in the choroid via the posterior ciliary arteries about 10–15 seconds after intravenous injection into the antecubital vein. Within the next 1–3 seconds, the dye appears in the retina via the central retinal artery. The dye then flows into the retinal arterioles, the capillaries, the retinal veins, and finally drains via the central retinal vein (Fig. 4A). A fluorescein angiogram might show fluorescein dye leakage from abnormal retinal vessels and new vessels, indicating breakdown of the BRB (Fig. 4B).
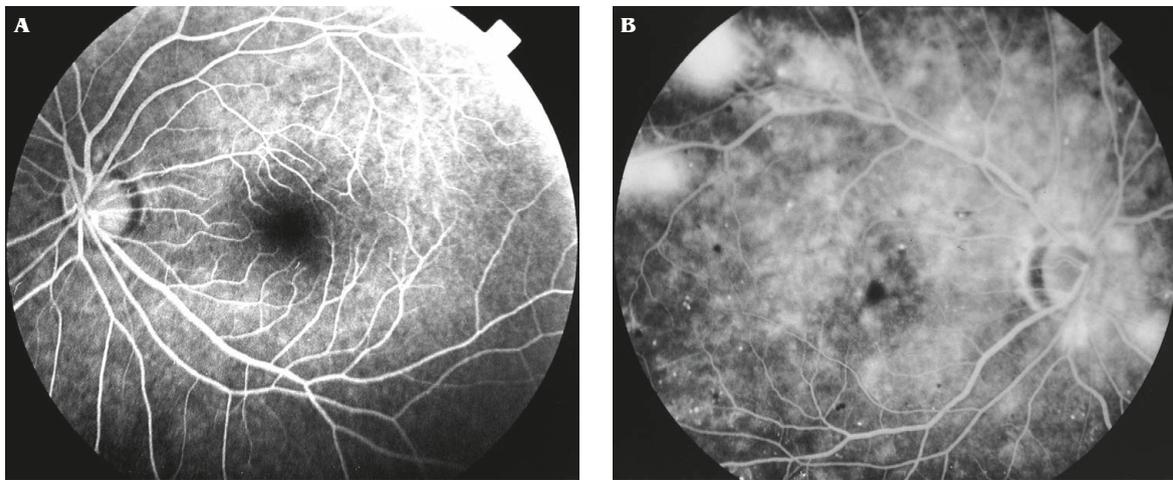
Fig. 4 — Fluorescein angiography: (A) normal fluorescein angiogram; (B) fluorescein dye leakage from retinal new vessels, indicating breakdown of the blood-retinal barrier.
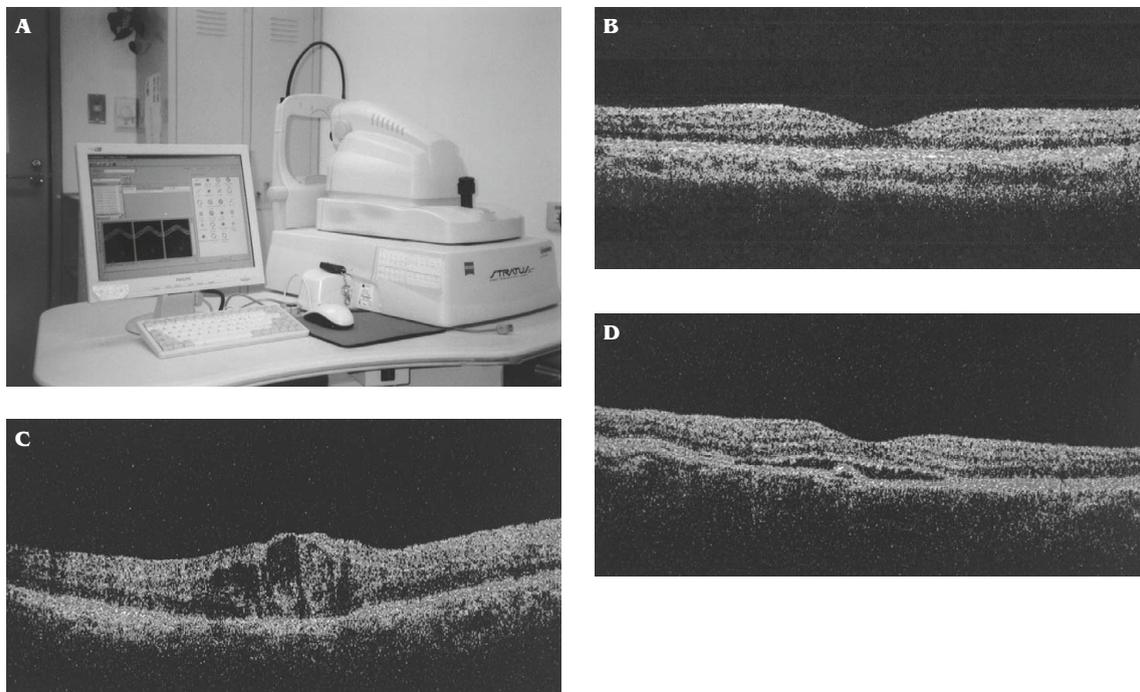

Fig. 5 — Optical coherence tomography: (A) equipment; (B) normal retina; (C) retinal edema in diabetic retinopathy, indicating breakdown of the inner blood-retinal barrier; (D) retinal edema in central serous chorioretinopathy manifested by serous detachment of the neurosensory retina and retinal pigment epithelium, indicating breakdown of the outer blood-retinal barrier.

3.4. Optical coherence tomography

Optical coherence tomography is a high-resolution, noninvasive imaging technique that can provide cross-sectional images of the retina. An interferometer detects echo time delays of light reflected from different layers of the retina and forms an optical cross-section of the retina (Figs. 5A and 5B). The optical beam is infrared light at 843 nm with a power of approximately 200 μW, which is well tolerated by the eye. The cross-sectional images have a 10-μm depth resolution in the retina. This technique can also be used to examine the optic disc, the peripapillary region, and the nerve fiber layer [11,12]. Optical coherence

tomography can also be used to detect retinal edema that originates from breakdown of the inner or outer BRB (Figs. 5C and 5D).

4. Basic research

Many basic studies have investigated morphological changes in the blood-ocular barriers. However, recent studies have focused on functional changes.

4.1. Mediators that influence function of blood-ocular barriers

The sites of breakdown of the BAB remain unknown, but are possibly the stroma of the iris root and the iris vessels [13]. Changes may be mediated by increased prostaglandin and lactate dehydrogenase levels [14,15], and may also be influenced by neurogenic or humoral factors [16].

The actions of prostaglandins include miosis, vasodilatation, pressure elevation, and increased permeability of the BAB. Previous reports suggested that dysfunction of the BAB may be minimized or eliminated by pre-treatment with a prostaglandin inhibitor, such as aspirin or indomethacin [14,15]. In studies of prostaglandin E_2-induced aqueous flare elevation, calcium channel blockers and cyclooxygenase inhibitors partly inhibited aqueous flare elevation due to their anti-inflammatory activity [17,18].

Intravitreal injection of interleukin (IL)-1 or IL-6 has been shown to induce a significant increase in aqueous flare values [19]. Among these, intravitreal injection of IL-1β has been noted to significantly increase the release of prostaglandin E_2 into the aqueous in rabbits [20,21]. Furthermore, IL-1 is induced by IL-6 in retinal pigment epithelial cells [22].

Vascular endothelial growth factor, which is also known as vascular permeability factor, is a specific growth factor for endothelial cells [23]. Murata et al demonstrated that vascular endothelial growth factor plays a role in the breakdown of the BRB in streptozotocin-induced diabetic rats [24].

4.2. Effects of retinal laser photocoagulation on blood-ocular barriers

Retinal laser photocoagulation has been widely used in the treatment of various retinal diseases. Peyman and coworkers demonstrated that in the acute stage of photocoagulation burns, the BRB is disrupted at the level of the retinal pigment epithelium [25].

Vitreous fluorophotometry studies of the effects of argon laser retinal photocoagulation and cryotherapy on the BRB in pigmented rabbits demonstrated that both modalities cause a significant breakdown in the BRB, which peaks 6 days after treatment; the barrier function returns to baseline values by 15 days post-treatment [26]. Inoue et al reported a significant increase in aqueous flare values on the first postoperative day following retinal photocoagulation, with the values returning to baseline by day 7 [19]. A study by our laboratory demonstrated significant disruption of the BAB 1 week after retinal laser photocoagulation. The aqueous flare value peaked on day 1, gradually decreased during the first week, and became insignificant by day 14 [27].

4.3. Intravitreal administration of anti-vascular endothelial growth factor agents

The role of vascular endothelial growth factor in many ocular diseases in which breakdown of the blood-ocular barriers and angiogenesis play major roles has been identified. These include diabetic retinopathy, age-related macular degeneration, retinal vein occlusion, and other diseases [28,29]. Inhibition of vascular endothelial growth factor activity by either pegaptanib (Macugen; Eyetech Pharmaceuticals Inc., New York, NY, USA) or intravitreal ranibizumab (Lucentis; Genentech Inc., South San Francisco, CA, USA) has been shown to be effective in the treatment of age-related macular degeneration [30,31].

Recently, there have been encouraging reports about off-label use of intravitreal bevacizumab (Avastin; Genentech Inc.) in the treatment of many ocular diseases. Bevacizumab is a full-length humanized murine monoclonal IgG antibody that binds to all isoforms of the vascular endothelial growth factor molecule [32,33]. However, intravitreal injection of a full length IgG antibody has the potential risk of an intraocular inflammatory response, and a recent study by our laboratory demonstrated that intravitreal injection of bevacizumab can produce breakdown of the BAB in rabbits.

5. Clinical applications

Many ocular diseases alter the function of the blood-ocular barriers. Some common ocular diseases involving blood-ocular barriers, or disease treatments that affect blood-ocular barriers, are described.

5.1. Medications

Some topical and systemic medications affect BAB function [34]. Medications that increase the aqueous flare intensity include pilocarpine [35],

beta-blockers (36), acetazolamide (37), mannitol (38) and apraclonidine (39). Topical application of the mydriatics tropicamide and phenylephrine results in a decrease in aqueous flare intensity. The magnitude of this change is approximately 10–20% in healthy subjects (34).

5.2.   Diabetic retinopathy

In recent decades, the number of individuals with diabetes has increased. In surveys performed in 1970, 1979 and 1986 in Taipei, the prevalence of diabetes was 5.05%, 7.10% and 8.17%, respectively. In rural areas of Taiwan, a prevalence of 5.06% was noted during a 1986 survey (40–42). Diabetic retinopathy has now become a major cause of loss of visual acuity in adults (43).

Diabetic retinopathy is generally associated with increased permeability of the blood-ocular barrier. Laser flare photometry demonstrated that abnormality of BAB function is correlated with the clinical grade of retinopathy. There was no significant difference between healthy subjects and diabetic subjects without retinopathy (44,45).

In some patients, vitreous fluorophotometry shows abnormality in BRB function before ophthalmoscopy can detect any abnormalities (6). Abnormalities in BRB function in diabetic subjects may be reversed with strict metabolic control (46). Some reports demonstrated that the vitreous fluorophotometric value is normal in diabetic subjects with no or minimal retinopathy, and is significantly higher in advanced retinopathy (47,48).

In Fig. 4B, fluorescein angiography shows marked fluorescein dye leakage from the optic disc and retinal new vessels in a patient with proliferative diabetic retinopathy. In Fig. 5C, optical coherence tomography shows cystoid spaces in the retina in a patient with diabetic retinopathy. These features demonstrate that this disease mainly affects the inner BRB.

5.3.   Retinitis pigmentosa and degenerative diseases

Retinitis pigmentosa is a group of hereditary diseases with a primary abnormality of the photoreceptor-retinal pigment epithelium complex. It is characterized by night blindness, loss of peripheral vision, retinal *bone-spicule* pigmentation, retinal arteriolar attenuation, and waxy optic disc pallor. Patients with retinitis pigmentosa have been shown to have abnormal BAB function, as indicated by increased aqueous flare intensity. In addition, subjects with typical retinitis pigmentosa have higher aqueous flare intensity than those with pericentral retinitis pigmentosa (49).

Patients with retinitis pigmentosa have also been shown to have abnormal BRB function, as assessed by vitreous fluorophotometry. Abnormal vitreous fluorophotometric recordings may precede ophthalmologically visible fundus changes (50).

Abnormal BAB function has also been detected with other retinal degenerative diseases, including crystalline retinopathy (51), retinitis punctata albecens (52), and reticular dystrophy of the retinal pigment epithelium (53). Among these, crystalline retinopathy also has associated abnormal BRB function (54).

5.4.   Age-related macular degeneration

Age-related macular degeneration is a common degenerative condition of the retina that may affect the central vision. It is divided into non-exudative and exudative types. Exudative age-related macular degeneration is characterized by the presence of choroidal neovascularization, which may lead to hemorrhage or lipid exudation.

Laser flare photometry has been used to examine various stages of age-related macular degeneration. The aqueous flare values increase with advancing age-related macular degeneration in both the non-exudative and exudative types (55). Vitreous fluorophotometry has demonstrated alteration in BRB function (56). Fluorescein angiography shows fluorescein dye leakage from the choroidal neovascular vessels. Optical coherence tomography shows fusiform thickening in the reflective band, consistent with a neovascular membrane.

5.5.   Ocular tumors

Malignant melanoma of the ciliary body or choroid may cause alterations in blood-ocular barriers. Laser flare photometry demonstrated that the aqueous flare values were higher in eyes with malignant melanoma than in normal controls. The researchers described the ability of laser flare photometry to differentiate melanoma from benign lesions with an exudative component (57). Another report demonstrated that aqueous flare values are related to tumor height and volume (58). A study by our laboratory demonstrated that combined hamartoma of the retina and retinal pigment epithelium might cause a breakdown in BAB function (59).

5.6.   Ocular inflammation

Laser flare photometry has been widely used to study BAB function in various types of uveitis. This method could be used to monitor the severity of

uveitis, to predict recurrence, and to monitor the effects of treatment. Increased aqueous flare values have been reported in patients with Behcet's disease (60), sarcoidosis (60), ocular toxoplasmosis (61), acute anterior uveitis (61), herpes zoster ophthalmicus (61), acquired immunodeficiency syndrome (62), and cytomegalovirus retinitis (62).

Laser flare photometry has also been applied to study levels of post-surgical inflammation of the eye. Aqueous flare values have been reported to increase after cataract surgery (63). Anti-inflammatory effects of diclofenac and fluorometholone on BAB function after cataract surgery have been reported (64). Alteration in BAB function has also been noted after retinal detachment surgery (65).

5.7. Central serous chorioretinopathy

Central serous chorioretinopathy is characterized by a circumscribed serous detachment of the neurosensory retina in the macula. An associated serous detachment of the retinal pigment epithelium may occur. Vitreous fluorophotometry has demonstrated abnormal BRB function. Abnormalities in vitreous fluorophotometric recordings were associated with the size and height of the retinal serous detachment and the stage of activity (56).

In unpublished work, our laboratory has used laser flare photometry to demonstrate abnormal BAB function in the acute stage of retinal serous detachment. Fluorescein angiography shows a spot of hyperfluorescence at the level of the retinal pigment epithelium. There is fluorescein dye pooling in the late phase of neurosensory detachment. Optical coherence tomography shows serous detachment of the neurosensory retina, and may have been associated with serous detachment of the retinal pigment epithelium (Fig. 5D). These features demonstrate that this disease mainly affects the outer BRB.

6. Conclusion

Blood-ocular barriers play important roles in the maintenance and function of the eye. Abnormal function in blood-ocular barriers may occur in various ocular diseases. Four current methods of examination, laser flare-cell photometry, vitreous fluorophotometry, fluorescein angiography and optical coherence tomography, provide excellent qualitative and quantitative evaluation of the function of the blood-ocular barriers. Further development of new techniques to provide more detailed analysis of the function of the blood-ocular barriers, and new medications for the management of abnormalities of the blood-ocular barriers, is encouraging.

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